TRAITE DE COOPERATION EN MATIERE DE BREVETS

Expéditeur: le BUREAU INTERNATIONAL

PCT

NOTIFICATION D'ELECTION

(règle 61.2 du PCT)

Destinataire:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

en sa qualité d'office élu

Date d'expédition (jour/mois/année) 22 novembre 2000 (22.11.00)	
Demande internationale no PCT/FR00/00456	**Référence du dossier du déposant ou du mandataire** B99/0640QT
Date du dépôt international (jour/mois/année) 24 février 2000 (24.02.00)	**Date de priorité** (jour/mois/année) 26 mars 1999 (26.03.99)
Déposant PLOS, Grégory	

1. L'office désigné est avisé de son élection qui a été faite:

[X] dans la demande d'examen préliminaire international présentée à l'administration chargée de l'examen préliminaire international le:

24 octobre 2000 (24.10.00)

[ ] dans une déclaration visant une élection ultérieure déposée auprès du Bureau international le:

2. L'élection [X] a été faite

[ ] n'a pas été faite

avant l'expiration d'un délai de 19 mois à compter de la date de priorité ou, lorsque la règle 32 s'applique, dans le délai visé à la règle 32.2b).

Bureau international de l'OMPI
34, chemin des Colombettes
1211 Genève 20, Suisse

no de télécopieur: (41-22) 740.14.35

Fonctionnaire autorisé

Diana Nissen

no de téléphone: (41-22) 338.83.38

INTERNATIONAL SEARCH REPORT

International Application No
PCT/FR 00/00456

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K7/13 A61K7/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 716 846 A (YAMAHATSU SANGYO KAISHA) 19 June 1996 (1996-06-19) page 1 -page 2, line 35 page 5, line 31-36 page 6 ---	1-26
Y	WO 97 19998 A (AASLYNG DORRIT ;NOVONORDISK AS (DK); ROERBAEK KAREN (DK); SOERENSE) 5 June 1997 (1997-06-05) page 1-8 claims 1-3,8,9,11-13 ---	1-26
A	EP 0 429 855 A (KAO CORP) 5 June 1991 (1991-06-05) the whole document ---	1-26

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 July 2000	14/07/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Sierra Gonzalez, M

Form PCT/ISA/210 (second sheet) (July 1992)

THIS PAGE BLANK

INTERNATIONAL SEARCH REPORT

Interr nal Application No
PCT/FR 00/00456

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 673 641 A (OREAL) 27 September 1995 (1995-09-27) the whole document	1-26

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

THIS PAGE BLANK

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interr ...nal Application No
PCT/FR 00/00456

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0716846 A	19-06-1996	AU 3662495 A	27-06-1996
		CA 2150596 A	17-06-1996
		JP 8217652 A	27-08-1996
WO 9719998 A	05-06-1997	AU 7691296 A	19-06-1997
		EP 0863950 A	16-09-1998
		JP 2000503042 T	14-03-2000
EP 0429855 A	05-06-1991	JP 1967510 C	18-09-1995
		JP 3141215 A	17-06-1991
		JP 6099290 B	07-12-1994
		AT 111344 T	15-09-1994
		DE 69012515 D	20-10-1994
		DE 69012515 T	12-01-1995
		US 5104413 A	14-04-1992
EP 0673641 A	27-09-1995	FR 2717383 A	22-09-1995
		CA 2145024 A	22-09-1995
		DE 69500058 D	14-11-1996
		DE 69500058 T	13-02-1997
		ES 2095780 T	16-02-1997
		JP 2582233 B	19-02-1997
		JP 7316029 A	05-12-1995
		US 5735908 A	07-04-1998

THIS PAGE BLANK

TRAITE DE COOPERATION EN MATIERE DE BREVETS

Expéditeur: le BUREAU INTERNATIONAL

PCT

AVIS INFORMANT LE DEPOSANT DE LA COMMUNICATION DE LA DEMANDE INTERNATIONALE AUX OFFICES DESIGNES

(règle 47.1.c), première phrase, du PCT)

Destinataire:
BUREAU D.A. CASALONGA JOSSE
8, avenue Percier
F-75008 Paris
FRANCE

CASALONGA
13 OCT. 2000

Date d'expédition (jour/mois/année) 05 octobre 2000 (05.10.00)	
Référence du dossier du déposant ou du mandataire B99/0640QT	**AVIS IMPORTANT**

Demande internationale no	**Date du dépôt international** (jour/mois/année)	**Date de priorité** (jour/mois/année)
PCT/FR00/00456	24 février 2000 (24.02.00)	26 mars 1999 (26.03.99)

Déposant
L'OREAL etc

1. Il est notifié par la présente qu'à la date indiquée ci-dessus comme date d'expédition de cet avis, le Bureau international a communiqué, comme le prévoit l'article 20, la demande internationale aux offices désignés suivants:
   AU,KP,KR,US

   Conformément à la règle 47.1.c), troisième phrase, ces offices acceptent le présent avis comme preuve déterminante du fait que la communication de la demande internationale a bien eu lieu à la date d'expédition indiquée plus haut, et le déposant n'est pas tenu de remettre de copie de la demande internationale à l'office ou aux offices désignés.

2. Les offices désignés suivants ont renoncé à l'exigence selon laquelle cette communication doit être effectuée à cette date:
   AE,AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,CA,CH,CN,CU,CZ,DE,DK,EA,EE,EP,ES,FI,GB,GD,GE,GH,
   GM,HR,HU,ID,IL,IN,IS,JP,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MN,MW,MX,NO,NZ,
   OA,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZW
   La communication sera effectuée seulement sur demande de ces offices. De plus, le déposant n'est pas tenu de remettre de copie de la demande internationale aux offices en question (règle 49.1)a-bis)).

3. Le présent avis est accompagné d'une copie de la demande internationale publiée par le Bureau international le
   05 octobre 2000 (05.10.00) sous le numéro WO 00/57848

RAPPEL CONCERNANT LE CHAPITRE II (article 31.2)a) et règle 54.2)

Si le déposant souhaite reporter l'ouverture de la phase nationale jusqu'à 30 mois (ou plus pour ce qui concerne certains offices) à compter de la date de priorité, **la demande d'examen préliminaire international** doit être présentée à l'administration compétente chargée de l'examen préliminaire international avant l'expiration d'un délai de 19 mois à compter de la date de priorité.

Il appartient exclusivement au déposant de veiller au respect du délai de 19 mois.

Il est à noter que seul un déposant qui est ressortissant d'un Etat contractant du PCT lié par le chapitre II ou qui y a son domicile peut présenter une demande d'examen préliminaire international.

RAPPEL CONCERNANT L'OUVERTURE DE LA PHASE NATIONALE (article 22 ou 39.1))

Si le déposant souhaite que la demande internationale procède en phase nationale, il doit, dans le délai de 20 mois ou de 30 mois, ou plus pour ce qui concerne certains offices, accomplir les actes mentionnés dans ces dispositions auprès de chaque office désigné ou élu.

Pour d'autres informations importantes concernant les délais et les actes à accomplir pour l'ouverture de la phase nationale, voir l'annexe du formulaire PCT/IB/301 (Notification de la réception de l'exemplaire original) et le volume II du Guide du déposant du PCT.

Bureau international de l'OMPI
34, chemin des Colombettes
1211 Genève 20, Suisse

no de télécopieur (41-22) 740.14.35

Fonctionnaire autorisé
J. Zahra

no de téléphone (41-22) 338.83.38

TRAITE DE COOPERATION EN MATIERE DE BREVETS

Expéditeur : le BUREAU INTERNATIONAL

PCT

NOTIFICATION RELATIVE A LA PRESENTATION OU A LA TRANSMISSION DU DOCUMENT DE PRIORITE

(instruction administrative 411 du PCT)

Destinataire:

BUREAU D.A. CASALONGA JOSSE
8, avenue Percier
F-75008 Paris
FRANCE

CASALONGA
21 AVR. 2000

Date d'expédition (jour/mois/année) 14 avril 2000 (14.04.00)	
Référence du dossier du déposant ou du mandataire B99/0640QT	**NOTIFICATION IMPORTANTE**
Demande internationale no PCT/FR00/00456	Date du dépôt international (jour/mois/année) 24 février 2000 (24.02.00)
Date de publication internationale (jour/mois/année) Pas encore publiée	Date de priorité (jour/mois/année) 26 mars 1999 (26.03.99)
Déposant L'OREAL etc	

1. La date de réception (sauf lorsque les lettres "NR" figurent dans la colonne de droite) par le Bureau international du ou des documents de priorité correspondant à la ou aux demandes énumérées ci-après est notifiée au déposant. Sauf indication contraire consistant en un astérisque figurant à côté d'une date de réception, ou les lettres "NR", dans la colonne de droite, le document de priorité en question a été présenté ou transmis au Bureau international d'une manière conforme à la règle 17.1.a) ou b).
2. Ce formulaire met à jour et remplace toute notification relative à la présentation ou à la transmission du document de priorité qui a été envoyée précédemment.
3. Un **astérisque(*)** figurant à côté d'une date de réception dans la colonne de droite signale un document de priorité présenté ou transmis au Bureau international mais de manière non conforme à la règle 17.1.a) ou b). Dans ce cas, **l'attention du déposant est appelée** sur la règle 17.1.c) qui stipule qu'aucun office désigné ne peut décider de ne pas tenir compte de la revendication de priorité avant d'avoir donné au déposant la possibilité de remettre le document de priorité dans un délai raisonnable en l'espèce.
4. Les **lettres "NR"** figurant dans la colonne de droite signalent un document de priorité que le Bureau international n'a pas reçu ou que le déposant n'a pas demandé à l'office récepteur de préparer et de transmettre au Bureau international, conformément à la règle 17.1.a) ou b), respectivement. Dans ce cas, **l'attention du déposant est appelée** sur la règle 17.1.c) qui stipule qu'aucun office désigné ne peut décider de ne pas tenir compte de la revendication de priorité avant d'avoir donné au déposant la possibilité de remettre le document de priorité dans un délai raisonnable en l'espèce.

Date de priorité	Demande de priorité n°	Pays, office régional ou office récepteur selon le PCT	Date de réception du document de priorité
26 mars 1999 (26.03.99)	99/03829	FR	27 mars 2000 (27.03.00)

Bureau international de l'OMPI
34, chemin des Colombettes
1211 Genève 20, Suisse

no de télécopieur (41-22) 740.14.35

Fonctionnaire autorisé:

Marc Salzman

no de téléphone (41-22) 338.83.38

TRAITE DE COOPERATION EN MATIERE DE BREVETS

Expéditeur: le BUREAU INTERNATIONAL

PCT

NOTIFICATION DE LA RECEPTION DE L'EXEMPLAIRE ORIGINAL

(règle 24.2.a) du PCT)

Destinataire:

BUREAU D.A. CASALONGA JOSSE
8, avenue Percier
F-75008 Paris
FRANCE

CASALONGA
- 7 AVR. 2000

Date d'expédition (jour/mois/année)	NOTIFICATION IMPORTANTE
29 mars 2000 (29.03.00)	
Référence du dossier du déposant ou du mandataire	**Demande internationale no**
B99/0640QT	PCT/FR00/00456

Il est notifié au déposant que le Bureau international a reçu l'exemplaire original de la demande internationale précisée ci-après.

Nom(s) du ou des déposants et de l'Etat ou des Etats pour lesquels ils sont déposants:

L'OREAL (pour tous les Etats désignés sauf US)
PLOS, Grégory (pour US seulement)

Date du dépôt international	:	24 février 2000 (24.02.00)
Date(s) de priorité revendiquée(s)	:	26 mars 1999 (26.03.99)
Date de réception de l'exemplaire original par le Bureau international	:	21 mars 2000 (21.03.00)
Liste des offices désignés	:	

AP :GH,GM,KE,LS,MW,SD,SL,SZ,TZ,UG,ZW
EA :AM,AZ,BY,KG,KZ,MD,RU,TJ,TM
EP :AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE
OA :BF,BJ,CF,CG,CI,CM,GA,GN,GW,ML,MR,NE,SN,TD,TG
National :AE,AL,AM,AT,AU,AZ,BA,BB,BG,BR,BY,CA,CH,CN,CU,CZ,DE,DK,EE,ES,FI,GB,GD,GE,GH,GM,HR,HU,ID,IL,IN,IS,JP,KE,KG,KP,KR,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MN,MW,MX,NO,NZ,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,US,UZ,VN,YU,ZA,ZW

ATTENTION

Le déposant doit soigneusement vérifier les indications figurant dans la présente notification. En cas de divergence entre ces indications et celles que contient la demande internationale, il doit aviser immédiatement le Bureau international.

En outre, l'attention du déposant est appelée sur les renseignements donnés dans l'annexe en ce qui concerne

[X] les délais dans lesquels doit être abordée la phase nationale

[X] la confirmation des désignations faites par mesure de précaution

[X] les exigences relatives aux documents de priorité.

Une copie de la présente notification est envoyée à l'office récepteur et à l'administration chargée de la recherche internationale.

Bureau international de l'OMPI
34, chemin des Colombettes
1211 Genève 20, Suisse

n° de télécopieur (41-22) 740.14.35

Fonctionnaire autorisé

Jocelyne Rey-Millet

n° de téléphone (41-22) 338.83.38

THIS PAGE BLANK (USP

09/926209 Translation

8

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference B99/0640QT	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/FR00/00456	International filing date (*day/month/year*) 24 February 2000 (24.02.00)	Priority date (*day/month/year*) 26 March 1999 (26.03.99)
International Patent Classification (IPC) or national classification and IPC A61K 7/13		
Applicant L'OREAL		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☒ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand 24 October 2000 (24.10.00)	Date of completion of this report 17 December 2001 (17.12.2001)
Name and mailing address of the IPEA/EP Facsimile No.	Authorized officer Telephone No.

THIS PAGE BLANK

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/FR00/00456

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed

☒ the description:

pages 1-17, as originally filed

pages _____, filed with the demand

pages _____, filed with the letter of _____

☒ the claims:

pages 1-26, as originally filed

pages _____, as amended (together with any statement under Article 19

pages _____, filed with the demand

pages _____, filed with the letter of _____

☐ the drawings:

pages _____, as originally filed

pages _____, filed with the demand

pages _____, filed with the letter of _____

☐ the sequence listing part of the description:

pages _____, as originally filed

pages _____, filed with the demand

pages _____, filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheets/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rule 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-26	**YES**
	Claims		**NO**
Inventive step (IS)	Claims	1-26	**YES**
	Claims		**NO**
Industrial applicability (IA)	Claims	1-26	**YES**
	Claims		**NO**

2. Citations and explanations

1. Document EP-A-0 716 846 appears to be the most relevant of the documents cited in the search report.

Said document describes a hair dye composition based on the use of an oxidizing enzyme, uricase (with the exception of any other enzyme), in combination with an oxidation base and uric acid. A reducing agent, the redox potential of which must be simultaneously more positive than that of the ascorbic acid and more negative than that of the uric acid, may be present, such as N-acetyl-cysteine.

Said reducing agent has an antioxidant action, yet without inhibiting the reactions necessary for dyeing.

2. The present application differs from said prior art in that:

- the oxidizing enzyme is a laccase;
- no related substrate is present.

3. Insofar as said prior art:

- does not teach or suggest the use of a laccase instead of a uricase; and,
- the selection of the reducing agent is determined by the redox potential of the substrate related to the enzyme,

the present application, based on the use of a laccase and N-acetyl-cysteine, cannot be considered to be obviously derivable from document FR-A-0 716 846,

unless it is considered that the uricase can be substituted by a laccase, but that the N-acetyl-cysteine must nevertheless be preserved; which is (i) a blatant contradiction to the teaching of said document (only the uricase is mentioned) and (ii) illogical (the N-acetyl cysteine selection is a function of the potential of the substrate related to the selected enzyme), which the present authority cannot do.

4. The applicant's aim, namely that of preserving the oxidation dye precursors and couplers without inhibiting the laccase action, has been convincingly achieved (see the results of the experiments).

5. The present authority therefore considers that the objective established by the applicant has been achieved in an original manner and is not derivable from the prior art; the requirements of PCT Article 33(3) therefore appear to be satisfied.

THIS PAGE BLANK

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

Contrary to the requirement of EPC Rule 27.1(b), the applicant has neither cited nor commented on document EP-A-0 716 846 in the description.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claim 2 specifies that at least one laccase is incorporated into the composition (A), whereas the description (on page 3, line 17) only refers to incorporating one laccase.

In Claims 17 and 18, the polymers have broader definitions than those found in the description (cf. the molecular weight on page 11, lines 3 and 19); the definitions of the claims are not, therefore, consistent with the description.

The wording of Claims 21 and 22 lacks clarity insofar as said claims refer to a part of the contents of the method claims; it would have been preferable to use extensive wording for the subject matter claimed therein, even if such a wording would apparently be "heavier".

Claim 23 is independent, but in fact relates to a step of the method claimed in Claim 2; insofar as an excessive number of the same type of claim should be avoided, said claim should have been redrafted as a dependent claim.

The wording of Claim 24 can be clearer, because it is in fact very close to the wording of Claim 2, and is characterized only in that the composition (A) is stored separately (which is already indicated in Claim 2)!

TRAITE DE COOPERATION EN MATIERE DE BREVETS

PCT

RAPPORT DE RECHERCHE INTERNATIONALE

(article 18 et règles 43 et 44 du PCT)

Référence du dossier du déposant ou du mandataire B99/0640QT	**POUR SUITE A DONNER** voir la notification de transmission du rapport de recherche internationale (formulaire PCT/ISA/220) et, le cas échéant, le point 5 ci-après	
Demande internationale n° PCT/FR 00/ 00456	Date du dépôt international *(jour/mois/année)* 24/02/2000	(Date de priorité (la plus ancienne) *(jour/mois/année)* 26/03/1999
Déposant L'OREAL et al.		

Le présent rapport de recherche internationale, établi par l'administration chargée de la recherche internationale, est transmis au déposant conformément à l'article 18. Une copie en est transmise au Bureau international.

Ce rapport de recherche internationale comprend 3 feuilles.

[X] Il est aussi accompagné d'une copie de chaque document relatif à l'état de la technique qui y est cité.

1. **Base du rapport**

   a. En ce qui concerne la **langue,** la recherche internationale a été effectuée sur la base de la demande internationale dans la langue dans laquelle elle a été déposée, sauf indication contraire donnée sous le même point.

   [ ] la recherche internationale a été effectuée sur la base d'une traduction de la demande internationale remise à l'administration.

   b. En ce qui concerne **les séquences de nucléotides ou d'acides aminés** divulguées dans la demande internationale (le cas échéant), la recherche internationale a été effectuée sur la base du listage des séquences :

   [ ] contenu dans la demande internationale, sous forme écrite.

   [ ] déposée avec la demande internationale, sous forme déchiffrable par ordinateur.

   [ ] remis ultérieurement à l'administration, sous forme écrite.

   [ ] remis ultérieurement à l'administration, sous forme déchiffrable par ordinateur.

   [ ] La déclaration, selon laquelle le listage des séquences présenté par écrit et fourni ultérieurement ne vas pas au-delà de la divulgation faite dans la demande telle que déposée, a été fournie.

   [ ] La déclaration, selon laquelle les informations enregistrées sous forme déchiffrable par ordinateur sont identiques à celles du listage des séquences présenté par écrit, a été fournie.

2. [ ] **Il a été estimé que certaines revendications ne pouvaient pas faire l'objet d'une recherche** (voir le cadre I).

3. [ ] **Il y a absence d'unité de l'invention** (voir le cadre II).

4. En ce qui concerne le **titre,**

   [X] le texte est approuvé tel qu'il a été remis par le déposant.

   [ ] Le texte a été établi par l'administration et a la teneur suivante:

5. En ce qui concerne **l'abrégé,**

   [X] le texte est approuvé tel qu'il a été remis par le déposant

   [ ] le texte (reproduit dans le cadre III) a été établi par l'administration conformément à la règle 38.2b). Le déposant peut présenter des observations à l'administration dans un délai d'un mois à compter de la date d'expédition du présent rapport de recherche internationale.

6. La figure **des dessins** à publier avec l'abrégé est la Figure n° _____

   [ ] suggérée par le déposant.

   [ ] parce que le déposant n'a pas suggéré de figure.

   [ ] parce que cette figure caractérise mieux l'invention.

   [ ] Aucune des figures n'est à publier.

RAPPORT DE RECHERCHE INTERNATIONALE

Demande Internationale No
PCT/FR 00/00456

A. CLASSEMENT DE L'OBJET DE LA DEMANDE
CIB 7 A61K7/13 A61K7/06

Selon la classification internationale des brevets (CIB) ou à la fois selon la classification nationale et la CIB

B. DOMAINES SUR LESQUELS LA RECHERCHE A PORTE

Documentation minimale consultée (système de classification suivi des symboles de classement)
CIB 7 A61K

Documentation consultée autre que la documentation minimale dans la mesure où ces documents relèvent des domaines sur lesquels a porté la recherche

Base de données électronique consultée au cours de la recherche internationale (nom de la base de données, et si réalisable, termes de recherche utilisés)
EPO-Internal, CHEM ABS Data, WPI Data, PAJ

C. DOCUMENTS CONSIDERES COMME PERTINENTS

Catégorie °	Identification des documents cités, avec, le cas échéant, l'indication des passages pertinents	no. des revendications visées
Y	EP 0 716 846 A (YAMAHATSU SANGYO KAISHA) 19 juin 1996 (1996-06-19) page 1 -page 2, ligne 35 page 5, ligne 31-36 page 6 ---	1-26
Y	WO 97 19998 A (AASLYNG DORRIT ;NOVONORDISK AS (DK); ROERBAEK KAREN (DK); SOERENSE) 5 juin 1997 (1997-06-05) page 1-8 revendications 1-3,8,9,11-13 ---	1-26
A	EP 0 429 855 A (KAO CORP) 5 juin 1991 (1991-06-05) le document en entier --- -/--	1-26

[X] Voir la suite du cadre C pour la fin de la liste des documents

[X] Les documents de familles de brevets sont indiqués en annexe

° Catégories spéciales de documents cités:

"A" document définissant l'état général de la technique, non considéré comme particulièrement pertinent

"E" document antérieur, mais publié à la date de dépôt international ou après cette date

"L" document pouvant jeter un doute sur une revendication de priorité ou cité pour déterminer la date de publication d'une autre citation ou pour une raison spéciale (telle qu'indiquée)

"O" document se référant à une divulgation orale, à un usage, à une exposition ou tous autres moyens

"P" document publié avant la date de dépôt international, mais postérieurement à la date de priorité revendiquée

"T" document ultérieur publié après la date de dépôt international ou la date de priorité et n'appartenant pas à l'état de la technique pertinent, mais cité pour comprendre le principe ou la théorie constituant la base de l'invention

"X" document particulièrement pertinent; l'inven tion revendiquée ne peut être considérée comme nouvelle ou comme impliquant une activité inventive par rapport au document considéré isolément

"Y" document particulièrement pertinent; l'inven tion revendiquée ne peut être considérée comme impliquant une activité inventive lorsque le document est associé à un ou plusieurs autres documents de même nature, cette combinaison étant évidente pour une personne du métier

"&" document qui fait partie de la même famille de brevets

Date à laquelle la recherche internationale a été effectivement achevée	Date d'expédition du présent rapport de recherche internationale
6 juillet 2000	14/07/2000

Nom et adresse postale de l'administration chargée de la recherche internationale	Fonctionnaire autorisé
Office Européen des Brevets, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Sierra Gonzalez, M

1

Formulaire PCT/ISA/210 (deuxième feuille) (juillet 1992)

THIS PAGE BLANK

RAPPORT DE RECHERCHE INTERNATIONALE

Demande Internationale No
PCT/FR 00/00456

C.(suite) DOCUMENTS CONSIDERES COMME PERTINENTS

Catégorie	Identification des documents cités, avec, le cas échéant, l'indicationdes passages pertinents	no. des revendications visées
A	EP 0 673 641 A (OREAL) 27 septembre 1995 (1995-09-27) le document en entier	1-26

1

THIS PAGE BLANK

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/FR 00/00456

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0716846 A	19-06-1996	AU 3662495 A	27-06-1996
		CA 2150596 A	17-06-1996
		JP 8217652 A	27-08-1996
WO 9719998 A	05-06-1997	AU 7691296 A	19-06-1997
		EP 0863950 A	16-09-1998
		JP 2000503042 T	14-03-2000
EP 0429855 A	05-06-1991	JP 1967510 C	18-09-1995
		JP 3141215 A	17-06-1991
		JP 6099290 B	07-12-1994
		AT 111344 T	15-09-1994
		DE 69012515 D	20-10-1994
		DE 69012515 T	12-01-1995
		US 5104413 A	14-04-1992
EP 0673641 A	27-09-1995	FR 2717383 A	22-09-1995
		CA 2145024 A	22-09-1995
		DE 69500058 D	14-11-1996
		DE 69500058 T	13-02-1997
		ES 2095780 T	16-02-1997
		JP 2582233 B	19-02-1997
		JP 7316029 A	05-12-1995
		US 5735908 A	07-04-1998

THIS PAGE BLANK

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C09B 67/00, A61K 7/13

A1

(11) International Publication Number: **WO 97/19998**

(43) International Publication Date: 5 June 1997 (05.06.97)

(21) International Application Number: PCT/DK96/00498

(22) International Filing Date: 29 November 1996 (29.11.96)

(30) Priority Data:
1356/95 30 November 1995 (30.11.95) DK

(71) Applicant *(for all designated States except US)*: NOVO NORDISK A/S [DK/DK]; Novo Allé, DK-2880 Bagsværd (DK).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: AASLYNG, Dorrit [DK/DK]; Novo Nordisk a/s, Novo Allé, DK-2880 Bagsværd (DK). SØRENSEN, Niels, Henrik [DK/DK]; Novo Nordisk a/s, Novo Allé, DK-2880 Bagsværd (DK). RØRBÆK, Karen [DK/DK]; Novo Nordisk a/s, Novo Allé, DK-2880 Bagsværd (DK).

(74) Common Representative: NOVO NORDISK A/S; Corporate Patents, Novo Allé, DK-2880 Bagsværd (DK).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: AN ENZYME FOR DYING KERATINOUS FIBRES

(57) Abstract

The present invention relates to a dyeing composition, a method for dying keratinous fibres, in particular hair, fur, hide and wool, and the use of a *Scytalidium* laccase for dyeing.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	**GB**	United Kingdom	**MW**	Malawi
AT	Austria	**GE**	Georgia	**MX**	Mexico
AU	Australia	**GN**	Guinea	**NE**	Niger
BB	Barbados	**GR**	Greece	**NL**	Netherlands
BE	Belgium	**HU**	Hungary	**NO**	Norway
BF	Burkina Faso	**IE**	Ireland	**NZ**	New Zealand
BG	Bulgaria	**IT**	Italy	**PL**	Poland
BJ	Benin	**JP**	Japan	**PT**	Portugal
BR	Brazil	**KE**	Kenya	**RO**	Romania
BY	Belarus	**KG**	Kyrgystan	**RU**	Russian Federation
CA	Canada	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CF	Central African Republic			**SE**	Sweden
CG	Congo	**KR**	Republic of Korea	**SG**	Singapore
CH	Switzerland	**KZ**	Kazakhstan	**SI**	Slovenia
CI	Côte d'Ivoire	**LI**	Liechtenstein	**SK**	Slovakia
CM	Cameroon	**LK**	Sri Lanka	**SN**	Senegal
CN	China	**LR**	Liberia	**SZ**	Swaziland
CS	Czechoslovakia	**LT**	Lithuania	**TD**	Chad
CZ	Czech Republic	**LU**	Luxembourg	**TG**	Togo
DE	Germany	**LV**	Latvia	**TJ**	Tajikistan
DK	Denmark	**MC**	Monaco	**TT**	Trinidad and Tobago
EE	Estonia	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**UG**	Uganda
FI	Finland	**ML**	Mali	**US**	United States of America
FR	France	**MN**	Mongolia	**UZ**	Uzbekistan
GA	Gabon	**MR**	Mauritania	**VN**	Viet Nam

Title: An enzyme for dying keratinous fibres

FIELD OF THE INVENTION

The present invention relates to a dyeing composition for keratinous fibres, in particular hair, fur, hide and wool, a method for dying and the use of a *Scytalidium* laccase for dyeing.

BACKGROUND OF THE INVENTION

It has been used for many years to dye the hair to cover appearing grey hair. The need to do so arises from the fact that grey hair is the first sign of having past adolescence, which can be hard to accept for many people.

For instance, in certain parts of Asia it is widely used by both men and women to dye the hair with dyes often referred to by humorous people as "black shoe polish".

During the last few decades hair dyeing has become more and more popular in the western world. At first Punk Rockers and other society critical groups dyed their hair in extreme colours as a part of their protest against the established society, but today especially many young people also uses hair dyes (in more soft tints than the Punk Rockers) as a sort of "cosmetic" to change or freshen up their "look".

Hair dyes

In general hair dyeing compositions on the market today can be divided into three main groups:

- temporary hair dyes,
- semi-permanent hair dyes, and
- permanent oxidative hair dyes.

The temporary hair dyes are only intended to change the natural hair colour for a short period of time and usually functions by depositing dyes on the surface of the hair. Such hair dyes are easy to remove with normal shampooing.

When using semi-permanent hair dyes the colour of the dyed hair can survive for five or more shampooings. This is achieved

by using dyes having a high affinity for hair keratin and which is able to penetrate into the interior of the hair shaft.

Permanent hair dyes are very durable to sunlight, shampooing and other hair treatments and need only to be refreshed once a month as new hair grows out. With these dyeing systems the dyes are created directly in and on the hair. Small aromatic colourless dye precursors (*e.g.* p-phenylene-diamine and o-aminophenol) penetrate deep into the hair where said dye precursors are oxidised by an oxidising agent into coloured polymeric compounds. These coloured compounds are larger than the dye precursors and can not be washed out of the hair.

By including compounds referred to as modifiers (or couplers) in the hair dyeing composition a number of hair colour tints can be obtained. Cathecol and Resorcinol are examples of such modifiers.

Traditionally H_2O_2 is used as the oxidizing agent (colour builder), but also as a bleaching agent. Dyeing compositions comprising H_2O_2 are often referred to as "lightening dyes" due to this lightening effect of H_2O_2.

The use of H_2O_2 in dye compositions have some disadvantages as H_2O_2 damages the hair. Further, oxidative dyeing often demands high pH (normally around pH 9-10), which also inflicts damage on the hair. Consequently, if using dye compositions comprising H_2O_2 it is not recommendable to dye the hair often.

To overcome the disadvantages of using H_2O_2 it has been suggested to use oxidation enzymes to replace H_2O_2.

US patent no. 3,251,742 (Revlon) describes a method for dyeing human hair by dye formation *in situ* (*i.e.* on the hair). An oxidative enzyme is used to the colour formation reactions at a substantially neutral pH (pH 7-8.5).

Laccases, tyrosinases, polyphenolases and catacolases are mentioned as the suitable oxidation enzymes.

EP patent no. 504.005 (Perma S.A.) concerns compositions for dying hair which do not require the presence of H_2O_2 (hydrogen peroxide). The composition comprises an enzyme capable of catalyzing the formation of the polymeric dyes and also dye precursors, such as bases and couplers, in a buffer solution wherein the pH of said composition is between 6.5 and 8 and

said enzyme has an optimal activity in the pH range between 6.5 and 8.

Rhizoctonia praticola laccase and *Rhus vernicifera* laccase have a pH-optimum between 6.5 and 8 and can be used to form the polymeric dyes according to this patent.

Abstract of Papers American Chemical Society vol. 209, no. 1-2, 1995 discloses the cloning of a laccase from a *Scytalidium thermophilum*. The abstract does not mention the use of said laccase for dyeing hair.

SUMMARY OF THE INVENTION

The object of the present invention is to provide improved permanent dyeing compositions for keratinous fibres, in particular hair, fur, hide and wool, which is less damaging to the keratinous fibres than *e.g.* dyeing compositions for hair using H_2O_2.

It has now surprisingly been found that it is possible to provide such an improved dyeing composition by using an enzyme derived from a strain of the filamentous fungus genus *Scytalidium* as the oxidation enzyme.

In the first aspect the invention relates to a permanent dyeing composition for keratinous fibres, in particular hair, fur, hide and wool, comprising an oxidation enzyme comprising

1) one or more oxidation enzymes derived from a strain of the genus *Scytalidium*,

2) one or more dye precursors, and

optionally 3) one or more modifiers.

In a preferred embodiment of the invention the oxidation enzyme is a laccase derived from a strain of the genus *Scytalidium*, in particular from a strain of the species *Scytalidium thermophilum*.

Secondly, it is the object of the invention to provide a method for dying keratinous fibres, comprising contacting a laccase derived from a strain of the genus Scytalidium with the keratinous fibres and at least one dye precursor in the presence or absence of at least one modifier for a suitable period of time and under conditions sufficient to permit oxidation of the dye precursor into a coloured compound.

Finally the invention relates to the use of an oxidation enzyme derived from a strain of the genus *Scytalidium* for oxidative dyeing of keratinous fibres, in particular hair, fur, hide and wool.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 shows the dyeing effect of the *Scytalidium thermophilum* laccase (rStL-FXu-1)

DETAILED DESCRIPTION OF THE INVENTION

The object of the present invention is to provide improved permanent dyeing compositions for keratinous fibres, in particular hair, fur, hide and wool, which is less damaging to the keratinous fibres than *e.g.* hair dyeing compositions using H_2O_2.

It has surprisingly be found that it is possible to provide such an improved dyeing composition by using an oxidation enzyme derived from a strain of the filamentous fungus genus *Scytalidium*.

When using said oxidation enzyme derived from a strain of the genus *Scytalidium* the colour developed is as wash stable as oxidative dyeing of *e.g.* hair using H_2O_2 and the light fastness is as good as when dyeing chemically.

Consequently, in the first aspect the present invention relates to a permanent dye composition for keratinous fibres , in particular hair, fur, hide and wool, comprising

1) one or more oxidation enzymes derived from a strain of the genus *Scytalidium*,

2) one or more dye precursors, and

optionally 3) one or more modifiers.

In an embodiment of the invention the oxidation enzyme is a laccase derived from a strain of genus *Scytalidium*, such as a strain of *Scytalidium thermophilum* *e.g.* the purified laccase described in WO 95/33837 (PCT/US95/06816) from Novo Nordisk, which is hereby incorporated. SEQ ID No 1 shows a DNA sequence encoding a suitable laccase derivable from a strain of the species *Scytalidium thermophilum*.

E. coli JM101 containing the expression vector pShTh15 comprising SEQ ID NO 1 has been deposited under the Budapest

Treaty with the Agricultural Research Service Patent Culture Collection, Northern Regional Research Center, 1815 University Street, Peoria, Illinois, 61604. The vector have been given the Accession Number NRRL B-21262.

Also contemplated according to the invention are laccases derived from other microorganisms being more than 80% homologous to SEQ ID NO 1 derived from a strain of the species *Scytalidium thermophilum*.

In addition, *Scytalidium* laccases also encompass alternative forms of laccases which may be found in *S. thermophilum* and as well as laccases which may be found in other fungi which are synonyms of fall within the definition of *S. thermophilum* as defined by Straatsma and Samson, (1993), Mycol. Res. 97, 321-328). These include *S. indonesiacum*, *Torula thermophila*, *Humicola brevis* var. thermoidea, *Humicola brevispora*, *H. grisea* var. thermoidea, *Humicola insolens*, and *Humicola lanuginosa* (also known as *Thermomyces lanuginosus*).

It is to be understood that the *Scytalidium* laccase may be produced homologously, or heterologously using filamentous fungus, yeast or bacteria as the host cell.

Examples of filamentous fungi host cells include strains of the species of *Trichoderma*, preferably a strain of *Trichoderma harzianum* or *Trichoderma reesei*, or a species of *Aspergillus*, most preferably *Aspergillus oryzae* or *Aspergillus niger*, or yeast cells, such as *e.g.* a strain of *Saccharomyces*, in particular *Saccharomyces cerevisiae*, *Saccharomyces kluyveri* or *Saccharomyces uvarum*, a strain of *Schizosaccharomyces* sp., such as *Schizosaccharomyces pombe*, a strain of *Hansenula* sp., *Pichia* sp., *Yarrowia* sp., such as *Yarrowia lipolytica*, or *Kluyveromyces* sp., such as *Kluyveromyces lactis*, or a bacteria, such as gram-positive bacteria such as strains of *Bacillus*, such as strains of *B. subtilis*, *B. licheniformis*, *B. lentus*, *B. brevis*, *B. stearothermophilus*, *B. alkalophilus*, *B. amyloliquefaciens*, *B. coagulans*, *B. circulans*, *B. lautus*, *B. megaterium* or *B. thuringiensis*, or strains of *Streptomyces*, such as *S. lividans* or *S. murinus*, or gram-negative bacteria such as *Escherichia coli*.

Laccases (benzenediol:oxygen oxidoreductases) (E.C. class

1.10.3.2 according to Enzyme Nomenclature (1992) Academic Press, Inc) are multi-copper containing enzymes that catalyze the oxidation of phenols. Laccase-mediated oxidations result in the production of aryloxy-radical intermediates from suitable phenolic substrates; the ultimate coupling of the intermediates so produced provides a combination of dimeric, oligomeric, and polymeric reaction products. Certain reaction products can be used to form dyes suitable for dyeing hair (see below).

In an embodiment of the invention the *Scytalidium* laccase is neutral. In the context of laccases of the present invention this means that the pH optimum lies in the range from between 6.0 and 8.0.

To obtain dyeing of the keratinous fibres, such as hair, the dyeing composition of the invention also comprises a dye precursor which is converted into a coloured compound (*i.e.* a dye) by the oxidation agent which according to the invention is an oxidation enzyme derived from a strain of the species *Scytalidium*, such as a strain of *Scytalidium thermophilum*.

Without being limited thereto the dye precursor(s) may be (an) aromatic compound(s) belonging to one of three major chemical families: the diamines, aminophenols (or aminonaphtols) and the phenols. Examples of isatin derivative dye precursors can be found in DE 4,314,317-A1. Further, a number of indole or indoline derivative dye precursors are disclosed in WO 94/00100. Said dye precursors mentioned in these documents are hereby incorporated herein by reference.

Examples of such suitable dye precursors include compounds from the group comprising p-phenylene-diamine (pPD), p-toluylene-diamine, chloro-p-phenylenediamine, p-aminophenol, o-aminophenol and 3,4-diaminotoluene, 2-methyl-1,4-diaminobenzene, 4-methyl-o-phenylenediamine, 2-methoxy-p-phenylenediamine, 2-chloro-1,4-diamino-benzene, 4-amino diphenylamine, 1-amino-4-β-methoxyethylamino-benzene, 1-amino-4-bis-(β-hydroxyethyl)-amonibenzene, 1-3-diamino-benzene, 2-methyl-1,3-diamino-benzene, 2,4-diaminotoluene, 2,6-diaminopyridine, 1-hydroxy-2-amino-benzene, 1-hydroxy-3-amino-benzene, 1-methyl-2-hydroxy-4-amino-benzene, 1-methyl-2-hydroxy-4-β-hydroxyethylamino-benzene, 1-

hydroxy-4-amino-ebnzene, 1-hydroxy-4-methylamino-benzene, 1-methoxy-2,4-diamino-benzene, 1-ethoxy-2,3-diamino-benzene, 1-β-hydroxyethyloxy-2,4-diamino-benzene, phenazines, such as 4,7-phenazinedicarboxylic acid, 2,7-phenazinedicarboxylic acid, 2-phenazinecarboxylic acid, 2,7-diaminophenazine, 2,8-diaminophenazine, 2,7-diamino-3,8-dimethoxyphenazine, 2,7-diamino-3-methoxyphenazine, 2,7-diamino 3-methoxyphenazine, 3-dimethyl 2,8-phenazinediamine, 2,2'-[(8-amino-7-methyl-2-phenazinyl)imino]bis-ethanol, 2,2'-[(8-amino-7-methoxy-2-phenazinyl)imino]bis-ethanol, 2,2'-[(8-amino-7-chloro-2-phenazinyl)imino]bis-ethanol, 2-[(8-amino-7-methyl-2-phenazinyl)amino]-ethanol, 2,2'-[(8-amino-2-phenazinyl)imino]bis-ethanol, 3-amino-7-(dimethylamino)-2,8-dimethyl-5-phenyl-chloride, 9-(diethylamino)- benzo[a]phenazine-1,5-diol, N-[8-(diethylamino)-2-phenazinyl]- methanesulfonamide, N-(8-methoxy-2-phenazinyl)- Methanesulfonamide, N,N,N',N'-tetramethyl-2,7-phenazinediamine, 3,7-dimethyl-2-phenazinamine, p-amino benzoic acids, such as p-amino benzoic acid ethyl, p-amino benzoic acid glycerid, p-amino benzoic acid isobutyl, p-dimethylamino benzoic acid amil, p-dimethylamino benzoic acid octyl, p-diethoxy amino benzoic amil, p- dipropoxy amino benzoic acid ethyl, acetylsalicylic acid, isatin derivatives, such as 2,3-diamino benzoic acid.

In an embodiment the laccase is used with the dye precursor directly to oxidise it into a coloured compound. The dye precursor may be used alone or in combination with other dye precursors.

However, it is believed that when using a diamine or an aminophenol as the dye precursor at least one of the intermediate in the copolymerisation must be an *ortho*- or *para*-diamine or aminophenol. Examples of such are described in US patent no. 3,251,742 (Revlon), the contents of which are incorporated herein by reference.

Optionally the dyeing composition of the invention (especially hair dyeing compositions) also comprises a modifier (coupler) by which a number of colour tints can be obtained. In general modifiers are used in hair dyeing compositions, as the colours resulting from hair dyeing compositions without modifier(s) are usually unacceptable for most people.

Modifiers are typically m-diamines, m-aminophenols, or polyphenols. The modifier (coupler) reacts with the dye precursor(s) in the presence of the oxidative enzyme, converting it into a coloured compound.

Examples of modifiers (couplers) include m-phenylenediamine, 2,4-diaminoanisole, 1-hydroxynaphthalene(α-naphthol), 1,4-dihydroxybenzene(hydroquinone), 1,5-dihydroxynapthalene, 1,2-dihydroxybenzene(pyrocatechol), 1,3-dihydroxybenzene (resorcinol), 1,3-dihydroxy-2-methylbenzene, 1,3-dihydroxy-4-chlorobenzene(4-chlororesorcinol), 1,2,3,trihydroxybenzene, 1,2,4-trihydroxybenzene, 1,2,4-trihydroxy-5-methylbenzene, 1,2,4-trihydroxytoluene.

In the second aspect the invention relates to a method for dying keratinous fibres, in particular hair, fur, hide and wool, comprising contacting a laccase derived from a strain of the genus *Scytalidium* with the keratinous fibres and at least one dye precursor in the presence or absence of at least one modifier, for a period of time and under conditions sufficient to permit oxidation of the dye precursor into coloured compounds (*i.e.* a dye).

The dyeing method can be conducted with one or more dye precursors, either alone or in combination with one or more modifiers.

The amount of dye precursor(s) and other ingredients used in the composition of the invention are in accordance with usual commercial amounts.

When using a *Scytalidium* laccase, such as the *Scytalidium thermophilum* laccase mentioned above, the method for dyeing keratinous fibres of the invention may be carried out at room temperature, preferably around the optimum temperature of the enzyme, at a pH in the range from 3.0 to 9.0, preferably 4.0 to 8.0, especially pH 6.0 to 8.0.

Suitable dye precursors and optional modifiers are described above.

The use of this *Scytalidium* laccase is an improvement over the more traditional use of H_2O_2 as the latter can damage the keratinous fibres, such as hair. Further, normally prior art methods requires a high pH, which is also damaging to the

keratinous fibres. In contrast hereto, the reaction with laccase can be conducted at acidic or neutral pH, and the oxygen needed for oxidation comes from the air, rather than via harsh chemical oxidation.

The result provided by the use of the *Scytalidium* laccase is comparable to that achieved with use of H_2O_2, not only in colour development, but also in wash stability and light fastness. An additional commercial advantage is that a single container package can be made containing both the laccase and the precursor, in an oxygen free atmosphere, which arrangement is not possible with the use of H_2O_2.

MATERIALS AND METHODS

Materials:

Hair:

6" De Meo Virgin Natural White Hair (De Meo Brothers Inc. US)

Enzymes:

Laccase from *Scytalidium thermophilum* described in WO 95/33837 (PCT/US95/06816) from Novo Nordisk

Deposit of Biological Material

The following biological material has been deposited on the 25th May 1994 under the terms of the Budapest Treaty with the Agricultural Research Service Patent Culture Collection, Northern Regional Research Center, 1815 University Street, Peoria, Illinois, 61604 and given the following accession number.

Deposit	Accession Number
E. coli JM101 containing pShTh15	NRRL B-21262.

Dye precursors:

0.1 % w/w p-phenylene-diamine in 0.1 M K-phosphate buffer, pH 7.0. (pPD)

0.1 % w/w p-toluylene-diamine in 0.1 M K-phosphate buffer, pH 7.0.

0.1 % w/w chloro-p-phenylenediamine in 0.1 M K-phosphate

buffer, pH 7.0.

0.1 % w/w p-aminophenol in 0.1 M K-phosphate buffer, pH 7.0.

0.1 % w/w o-aminophenol in 0.1 M K-phosphate buffer, pH 7.0.

0.1 % w/w 3,4-diaminotoluene in 0.1 M K-phosphate, buffer pH 7.0.

Modifiers:

0.1 % w/w m-phenylene-diamine in 0.1 M K-phosphate buffer, pH 7.0.

0.1 % w/w 2,4-diaminoanisole in 0,1 M K-phosphate buffer, pH 7.0.

0.1 % w/w a-naphthol in 0.1 M K-phosphate buffer, pH 7.0.

0.1 % w/w hydroquinone in 0.1 M K-phosphate buffer, pH 7.0.

0.1 % w/w pyrocatechol in 0.1 M K-phosphate buffer, pH 7.0.

0.1% w/w resorcinol in 0.1 M K-phosphate buffer, pH 7.0.

0.1 % w/w 4-chlororesorcinol in 0.1 M K-phosphate buffer, pH 7.0.

The dye precursor is combined with one of the above indicated modifiers so that the final concentration in the dyeing solution is 0.1 % w/w with respect to precursor and 0.1 % w/w with respect to modifier.

Other solutions:

3% H_2O_2 (in the final dye solution)

Commercial shampoo

Equipment:

Minolta CR200 Chroma Meter

Day light bulb: 1000 LUX (D65)

Determination of Laccase Activity (LACU)

Laccase activity is determined from the oxidation of syringaldazin under aerobic conditions. The violet colour produced is photometered at 530 nm. The analytical conditions are 19 mM syringaldazin, 23.2 mM acetate buffer, pH 5.5, 30°C, 1 min. reaction time.

1 laccase unit (LACU) is the amount of enzyme that catalyses

the conversion of 1.0 micromole syringaldazin per minute at these conditions.

Assessment of the hair colour

The quantitative colour of the hair tresses are determined on a Minolta CR200 Chroma Meter by the use the parameters L* ("0"=black and "100"=white), a* ("-"=green and "+"=red) and b* ("-" blue and "+" yellow).

DL*, Da* and Db* are the delta values of L*, a* and b* respectively compared to L*, a* and b* of untreated hair (e.g. $DL^* = L^*_{sample} - L^*_{untreated\ hair}$).

DE* is calculated as $DE^* = \sqrt{(DL^{*2} + Da^{*2} + Db^*)}$ and is an expression for the total quantitative colour change.

EXAMPLES

Example 1

Dyeing effect

The dyeing effect of a *Scytalidium thermophilum* laccase was tested using the dye precursor o-aminophenol and the modifier m-phenylenediamine.

Hair dyeing

1 gram De Meo white hair tresses were used.

4 ml dye precursor solution (including modifier) is mixed with 1 ml laccase on a Whirley mixer, applied to the hair tresses and incubated at 30°C for 60 minutes.

The hair tresses are then rinsed with running water, washed with shampoo, rinsed with running water, combed, and air dried.

The a*, b* and L* was determined on the Chroma Meter and the DE* values were then calculated.

A hair tress sample treated without enzyme was used as a blind.

The result of the hair dyeing test is shown in figure 1.

Example 2

Wash stability

Tresses of white De Meo hair (1 gram) is used for testing the wash stability of hair dyed using *Scytalidium thermophilum* laccase, compared with hair dyed using H_2O_2, and p-phenylene-diamine (pPD) as the dye precursor. Further the wash stability is compared with a commercial oxidative dye.

The oxidative hair dyeing is carried out as described in Example 1.

Hair wash

The dyed hair tresses are wetted and washed for 15 seconds with 50 ml of commercial shampoo, and rinsed with water for 1 minute and air dried. The hair tresses are washed up to 18 times.

The a*, b* and L* is determined om the Chroma Meter and the ΔE^* values are then calculated.

Example 3

The light fastness

Tresses of blond European hair are used for testing the light fastness of hair dyed using *Scytalidium thermophilum* laccase in comparison to hair dyed using H_2O_2. p-phenylene-diamine was used as dye precursor.

The dyeing of the hair was carried out as described in Example 1.

One hair tress is kept dark, while an other is kept at day light (*i.e.* under a day light bulb (D65)), at approximately 1000 LUX) for up to 275 hours.

The a*, b* and L* parameters are determined immediately after the dyeing of the hair, and further during exposure to day light.

DE* then calculated from the determined a*, b* and L* values.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Novo Nordisk A/S
(B) STREET: Novo Alle
(C) CITY: Bagsvaerd
(D) COUNTRY: Denmark
(E) POSTAL CODE (ZIP): DK-2880
(F) TELEPHONE: +45 4444 8888
(G) TELEFAX: +45 4449 3256

(ii) TITLE OF INVENTION: An enzyme for dying hair

(iii) NUMBER OF SEQUENCES: 2

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2476 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Scytalidium thermophilum

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 349..411

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 502..559

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 632..686

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 1739..1804

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: join (106..348, 412..501, 560..631, 687..1738, 1805..2194)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
CTGAATTTAA ATACAGGAAG ATCGCATTCA ATCCAGCCTA GACTGCACAA TGGTTCTGCA      60

CGACCGTCGC ACACCTGCCA ATAGTGTTAA TAACGGNCTA ATACC ATG AAG CGC TTC     117
                                                  Met Lys Arg Phe
                                                  1

TTC ATT AAT AGC CTT CTG CTT CTC GCA GGG CTC CTC AAC TCA GGG GCC       165
Phe Ile Asn Ser Leu Leu Leu Leu Ala Gly Leu Leu Asn Ser Gly Ala
5                   10                  15                  20

CTC GCG GCT CCG TCT ACA CAT CCC AGA TCA AAC CCC GAC ATA CTG CTT       213
```

```
Leu Ala Ala Pro Ser Thr His Pro Arg Ser Asn Pro Asp Ile Leu Leu
                  25                  30                  35

GAA AGA GAT GAC CAC TCC CTT ACG TCT CGG CAA GGT AGC TGT CAT TCT      261
Glu Arg Asp Asp His Ser Leu Thr Ser Arg Gln Gly Ser Cys His Ser
              40                  45                  50

CCA AGC AAC CGC GCC TGT TGG TGC TCT GGC TTC GAT ATC AAC ACG GAT      309
Pro Ser Asn Arg Ala Cys Trp Cys Ser Gly Phe Asp Ile Asn Thr Asp
          55                  60                  65

TAT GAG ACC AAG ACT CCA AAC ACC GGA GTG GTG CGG CGG GTTAGTATCC      358
Tyr Glu Thr Lys Thr Pro Asn Thr Gly Val Val Arg Arg
      70                  75                  80

CAAGTTACGT TTGACCAAGA AATGGACGTG AAGTGTGCTG ACTCTCCCGC TAG          411

TAC ACC TTT GAT ATC ACC GAA GTC GAC AAC CGC CCC GGT CCC GAT GGG      459
Tyr Thr Phe Asp Ile Thr Glu Val Asp Asn Arg Pro Gly Pro Asp Gly
              85                  90                  95

GTC ATC AAG GAG AAG CTC ATG CTT ATC AAC GAC AAA CTC CTG GTAGG       506
Val Ile Lys Glu Lys Leu Met Leu Ile Asn Asp Lys Leu Leu
          100                 105                 110

GTCCTCTCGA ACGCCTGCGT CTGCCACACA GCGTAAAACT AACGAACCGC TAG          559

GGC CCG ACA GTC TTC GCA AAC TGG GGC GAC ACC ATC GAG GTG ACC GTC      607
Gly Pro Thr Val Phe Ala Asn Trp Gly Asp Thr Ile Glu Val Thr Val
              115                 120                 125

AAC AAC CAC CTG AGA ACC AAC GGA GTAAGCGTTC GGACACAAAG CCCAGCAACC    661
Asn Asn His Leu Arg Thr Asn Gly
          130                 135

TAGACACACT CAACTGACCA AGTAG ACC TCC ATC CAC TGG CAC GGC TTG CAC CAA  716
                            Thr Ser Ile His Trp His Gly Leu His Gln
                                140                             145

AAA GGA ACC AAC TAC CAC GAC GGC GCC AAC GGC GTG ACC GAG TGT CCC      764
Lys Gly Thr Asn Tyr His Asp Gly Ala Asn Gly Val Thr Glu Cys Pro
                  150                 155                 160

ATC CCG CCC GGT GGC TCC CGA GTC TAC AGC TTC CGA GCG CGC CAA TAT      812
Ile Pro Pro Gly Gly Ser Arg Val Tyr Ser Phe Arg Ala Arg Gln Tyr
              165                 170                 175

GGA ACG TCA TGG TAC CAC TCC CAC TTC TCC GCC CAG TAT GGC AAC GGC      860
Gly Thr Ser Trp Tyr His Ser His Phe Ser Ala Gln Tyr Gly Asn Gly
          180                 185                 190

GTG AGC GGC GCC ATC CAG ATC AAC GGA CCC GCC TCC CTG CCC TAC GAC      908
Val Ser Gly Ala Ile Gln Ile Asn Gly Pro Ala Ser Leu Pro Tyr Asp
      195                 200                 205

ATC GAC CTC GGC GTC CTC CCG CTG CAG GAC TGG TAC TAC AAG TCC GCC      956
Ile Asp Leu Gly Val Leu Pro Leu Xaa Asp Trp Tyr Tyr Lys Ser Ala
210                 215                 220                 225

GAC CAG CTC GTC ATC GAG ACC CTG GCC AAG GGC AAC GCT CCG TTC AGC     1004
Asp Gln Leu Val Ile Glu Thr Leu Xaa Lys Gly Asn Ala Pro Phe Ser
                  230                 235                 240

GAC AAC GTC CTC ATC AAC GGC ACC GCA AAG CAC CCC ACC ACT GGC GAA     1052
Asp Asn Val Leu Ile Asn Gly Thr Ala Lys His Pro Thr Thr Gly Glu
              245                 250                 255

GGG GAG TAC GCC ATC GTG AAG CTC ACC CCG GGC AAA CGC CAT CGC CTG     1100
Gly Glu Tyr Ala Ile Val Lys Leu Thr Pro Asp Lys Arg His Arg Leu
```

```
                260                 265                 270

CGG CTC ATC AAC ATG TCG GTG GAG AAC CAC TTC CAG GTC TCG CTG GCG      1148
Arg Leu Ile Asn Met Ser Val Glu Asn His Phe Gln Val Ser Leu Ala
    275                 280                 285

AAG CAC ACC ATG ACG GTC ATC GCG GCG GAC ATG GTC CCC GTC AAC GCC      1196
Lys His Thr Met Thr Val Ile Ala Ala Asp Met Val Pro Val Asn Ala
290                 295                 300                 305

ATG ACC GTC GAC AGC CTG TTT ATG GCC GNC GGG CAG CGG TAT GAT GTT      1244
Met Thr Val Asp Ser Leu Phe Met Ala Val Gly Gln Arg Tyr Asp Val
                310                 315                 320

ACC ATC GAC GCG AGC CAG GCG GTG GGG AAT TAC TGG TTC AAC ATC ACC      1292
Thr Ile Asp Ala Ser Gln Ala Val Gly Asn Tyr Trp Phe Asn Ile Thr
            325                 330                 335

TTT GGA GGG CAG CAG AAG TGC GGC TTC TCG CAC AAT CCG GCG CCG GCA      1340
Phe Gly Gly Gln Gln Lys Cys Gly Phe Ser His Asn Pro Ala Pro Ala
        340                 345                 350

GCC ATC TTT CGC TAC GAG GGC GCT CCT GAC GCT CTG CCG ACG GAT CCT      1388
Ala Ile Phe Arg Tyr Glu Gly Ala Pro Asp Ala Leu Pro Thr Asp Pro
    355                 360                 365

GGC GCT GCG CCA AAG GAT CAT CAG TGC CTG GAC ACT TTG GAT CTT TCA      1436
Gly Ala Ala Pro Lys Asp His Gln Cys Leu Asp Thr Leu Asp Leu Ser.
370                 375                 380                 385

CCG GTG GTG CAA AAG AAC GTG CCG GTT GAC GGG TTC GTC AAA GAG CCT      1484
Pro Val Val Gln Lys Asn Val Pro Val Asp Gly Phe Val Lys Glu Pro
                390                 395                 400

GGC AAT ACG CTG CCG GTG ACG CTC CAT GTT GAC CAG GCC GCG GCT CCA      1532
Gly Asn Thr Leu Pro Val Thr Leu His Val Asp Gln Ala Ala Ala Pro
            405                 410                 415

CAC GTG TTT ACG TGG AAG ATC AAC GGG AGC GCT GCG GAC GTG GAC TGG      1580
His Val Phe Thr Trp Lys Ile Asn Gly Ser Ala Ala Asp Val Asp Trp
        420                 425                 430

GAC AGG CCG GTG CTG GAG TAT GTC ATG AAC AAT GAC CTG TCT AGC ATT      1628
Asp Arg Pro Val Leu Glu Tyr Val Met Asn Asn Asp Leu Ser Ser Ile
    435                 440                 445

CCG GTC AAG AAC AAC ATT GTG AGG GTG GAC GGA GTC AAC GAG TGG ACG      1676
Pro Val Lys Asn Asn Ile Val Arg Val Asp Gly Val Asn Glu Trp Thr
450                 455                 460                 465

TAC TGG CTC GTC GAA AAC GAC CCG GAG GGC CGC CTC AGT TTG CCG CAT      1724
Tyr Trp Leu Val Glu Asn Asp Pro Glu Gly Arg Leu Ser Leu Pro His
                470                 475                 470

CCG ATG CAT CTA CAC GTAAGTCACA TCCCCCACTA CCATTCGGAA TGACCACCAG      1779
Pro Met His Leu His
            475

GTACTGACAC CCTCCTCCTC AATAG GGA CAC GAT TTC TTT GTC CTA GGC CGC      1831
                           Gly His Asp Phe Phe Val Leu Gly Arg
                           480                 485

TCC CCC GAC GTC TCG CCC GAT TCA GAA ACC CGC TTC GTC TTT GAC CCG      1879
Ser Pro Asp Val Ser Pro Asp Ser Glu Thr Arg Phe Val Phe Asp Pro
                490                 495                 500

GCC GTC GAC CTC CCC CGT CTG CGC GGA CAC AAC CCC GTC CGG CGC GAC      1927
Ala Val Asp Leu Pro Arg Leu Arg Gly His Asn Pro Val Arg Arg Asp
            505                 510                 515
```

```
GTC ACC ATG CTT CCC GCG CGC GGC TGG CTG CTG CTG GCC TTC CGC ACG      1975
Val Thr Met Leu Pro Ala Arg Glu Trp Leu Leu Leu Ala Phe Arg Thr
        520                 525                 530

GAC AAC CCG GGC GCG TGG TTG TTC CAC TGC CAC ATC GCG TGR CAC GTG      2023
Asp Asn Pro Gly Ala Trp Leu Phe His Cys His Ile Ala Trp His Val
    535                 540                 545

TCG GGC GGG TTA AGC GTC GAC TTT CTG GAG CGG CCG GAC GAG CTG CGC      2071
Ser Gly Gly Leu Ser Val Asp Phe Leu Glu Arg Pro Asp Glu Leu Arg
550                 555                 560                 565

GGG CAG CTG ACG GGA GAG AGC AAG GCG GAG TTG GAG CGT GTT TGT CGC      2119
Gly Gln Leu Thr Gly Glu Ser Lys Ala Glu Leu Glu Arg Val Cys Arg
                570                 575                 580

GAG TGG AAG GAT TGG GAG GCG AAG AGC CCG CAT GGG AAG ATC GAT TCG      2167
Glu Trp Lys Asp Trp Glu Ala Lys Ser Pro His Gly Lys Ile Asp Ser
            585                 590                 595

GGG TTG AAG CAG CGG CGA TGG GAT GCG TGAGGTAGTT GGGCGGATTG           2214
Gly Leu Lys Gln Arg Arg Trp Asp Ala
        600                 605

TTTAACACGT AGTGGGTAAG GTTGGGGCGG GTTTGTTTGG CGTTTTCAGG GGTTGGGGTG   2274

CGGATGCTGG TCATCCGGGA AACGGCTCTA CAACTGGTGT CAATAGACTA ATATAGAGTG   2334

ATCAAAGAAC TGAGGTTCTG AAAGAGGCGT GGAAGTCGCG TTGTGACTCC CTTTGCCATG   2394

TTGGGAAGTG TGGCTCAACA TTGTGTTCAG GTTTGCTCAG GGTGATNTCG AACTGACGTN   2454

TTGATGAGGG TTATTGCNTA GA                                            2476
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 616 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Scytalidium thermophilum

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
Met Lys Arg Phe Phe Ile Asn Ser Leu Leu Leu Leu Ala Gly Leu Leu
1               5                  10                  15

Asn Ser Gly Ala Leu Ala Ala Pro Ser Thr His Pro Arg Ser Asn Pro
            20                  25                  30

Asp Ile Leu Leu Glu Arg Asp Asp His Ser Leu Thr Ser Arg Gln Gly
        35                  40                  45

Ser Cys His Ser Pro Ser Asn Arg Ala Cys Trp Cys Ser Gly Phe Asp
    50                  55                  60

Ile Asn Thr Asp Tyr Glu Thr Lys Thr Pro Asn Thr Gly Val Val Arg
65                  70                  75                  80

Arg Tyr Thr Phe Asp Ile Thr Glu Val Asp Asn Arg Pro Gly Pro Asp
                85                  90                  95
```

Gly Val Ile Lys Glu Lys Leu Met Leu Ile Asn Asp Lys Leu Leu Gly
100 105 110

Pro Thr Val Phe Ala Asn Trp Gly Asp Thr Ile Glu Val Thr Val Asn
115 120 125

Asn His Leu Arg Thr Asn Gly Thr Ser Ile His Trp His Gly Leu His
130 135 140

Gln Lys Gly Thr Asn Tyr His Asp Gly Ala Asn Gly Val Thr Glu Cys
145 150 155 160

Pro Ile Pro Pro Gly Gly Ser Arg Val Tyr Ser Phe Arg Ala Arg Gln
165 170 175

Tyr Gly Thr Ser Trp Tyr His Ser His Phe Ser Ala Gln Tyr Gly Asn
180 185 190

Gly Val Ser Gly Ala Ile Gln Ile Asn Gly Pro Ala Ser Leu Pro Tyr
195 200 205

Asp Ile Asp Leu Gly Val Leu Pro Leu Gln Asp Trp Tyr Tyr Lys Ser
210 215 220

Ala Asp Gln Leu Val Ile Glu Thr Leu Ala Lys Gly Asn Ala Pro Phe
225 230 235 240

Ser Asp Asn Val Leu Ile Asn Gly Thr Ala Lys His Pro Thr Thr Gly
245 250 255

Glu Gly Glu Tyr Ala Ile Val Lys Leu Thr Pro Asp Lys Arg His Arg
260 265 270

Leu Arg Leu Ile Asn Met Ser Val Glu Asn His Phe Gln Val Ser Leu
275 280 285

Ala Lys His Thr Met Thr Val Ile Ala Ala Asp Met Val Pro Val Asn
290 295 300

Ala Met Thr Val Asp Ser Leu Phe Met Ala Xaa Gly Gln Arg Tyr Asp
305 310 315 320

Val Thr Ile Asp Ala Ser Gln Ala Val Gly Asn Tyr Trp Phe Asn Ile
325 330 335

Thr Phe Gly Gly Gln Gln Lys Cys Gly Phe Ser His Asn Pro Ala Pro
340 345 350

Ala Ala Ile Phe Arg Tyr Glu Gly Ala Pro Asp Ala Leu Pro Thr Asp
355 360 365

Pro Gly Ala Ala Pro Lys Asp His Gln Cys Leu Asp Thr Leu Asp Leu
370 375 380

Ser Pro Val Val Gln Lys Asn Val Pro Val Asp Gly Phe Val Lys Glu
385 390 395 400

Pro Gly Asn Thr Leu Pro Val Thr Leu His Val Asp Gln Ala Ala Ala
405 410 415

Pro His Val Phe Thr Trp Lys Ile Asn Gly Ser Ala Ala Asp Val Asp
420 425 430

Trp Asp Arg Pro Val Leu Glu Tyr Val Met Asn Asn Asp Leu Ser Ser
435 440 445

Ile Pro Val Lys Asn Asn Ile Val Arg Val Asp Gly Val Asn Glu Trp
450 455 460

```
Thr Tyr Trp Leu Val Glu Asn Asp Pro Glu Gly Arg Leu Ser Leu Pro
465                 470                 475                 480

His Pro Met His Leu His Gly His Asp Phe Phe Val Leu Gly Arg Ser
                485                 490                 495

Pro Asp Val Ser Pro Asp Ser Glu Thr Arg Phe Val Phe Asp Pro Ala
            500                 505                 510

Val Asp Leu Pro Arg Leu Arg Gly His Asn Pro Val Arg Arg Asp Val
        515                 520                 525

Thr Met Leu Pro Ala Arg Gly Trp Leu Leu Leu Ala Phe Arg Thr Asp
    530                 535                 540

Asn Pro Gly Ala Trp Leu Phe His Cys His Ile Ala Trp His Val Ser
545                 550                 555                 560

Gly Gly Leu Ser Val Asp Phe Leu Glu Arg Pro Asp Glu Leu Arg Gly
                565                 570                 575

Gln Leu Thr Gly Glu Ser Lys Ala Glu Leu Glu Arg Val Cys Arg Glu
            580                 585                 590

Trp Lys Asp Trp Glu Ala Lys Ser Pro His Gly Lys Ile Asp Ser Gly
        595                 600                 605

Leu Lys Gln Arg Arg Trp Asp Ala
    610                 615
```

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13 *bis*)

A. The indications made below relate to the microorganism referred to in the description on page 9, line 21-31.

B. IDENTIFICATION OF — Further deposits are identified on an additional sheet ☐

Name of depository institution
Agricultural Research Service Patent Culture Collection (NRRL)

Address of depository institution (*including postal code and country*)

Northern Regional Research Center
1815 University Street
Peoria, IL 61604, US

Date of deposit	Accession Number
25 May 1994	NRRL B-21262

C. ADDITIONAL INDICATIONS (*leave blank if not applicable*) — This information is continued on an additional sheet ☐

In respect of those designations in which a European and/or Australia Patent is sought, during the pendency of the patent application, a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (Rule 28(4) EPC/Regulation 3.25 of Australia Statutory Rule 1991 No. 71).

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (*if the indications are not for all designated States*)

E. SEPARATE FURNISHING OF INDICATIONS (*leave blank if not applicable*)

The indication listed below will be submitted to the International Bureau Later (*specify the general nature of the indications e.g. "Accession Number of Deposit"*)

For receiving Office use only	For International Bureau use only
☐ This sheet was received with the international application	☐ This sheet was received with the International Bureau on:
Authorized officer	Authorized officer

Form PCT/RO/134 (July 1992)

SUBSTITUTE SHEET

PATENT CLAIMS

1. A dyeing composition comprising an oxidation enzyme characterised in that the composition comprises:
1) one or more oxidation enzymes derived from a strain of the genus *Scytalidium*,
2) one or more dye precursors, and
optionally 3) one or more modifiers.

2. The dyeing composition according to claim 1, wherein the oxidation enzyme is derived from a strain of the genus *Scytalidium* laccase

3. The dyeing composition according to claim 2, wherein the laccase is derived from a strain of the species *Scytalidium thermophilum*.

4. The dyeing composition according to claims 2 and 3, wherein the laccase is neutral.

5. The dyeing composition according to claim 3, having the sequence shown in SEQ ID No 1.

6. The dyeing composition according to claim 5, wherein the sequence encoding the laccase is homologous to the SEQ ID NO 1.

7. The dyeing composition according to claim 6, wherein the sequence encoding the laccase is more than 80% homologous to SEQ ID NO 1.

8. The dyeing composition according to any of claims 1 to 7, comprising a dye precursor selected from the group comprising p-phenylene-diamine (pPD), p-toluylene-diamine, chloro-p-phenylenediamine, p-aminophenol, o-aminophenol and 3,4-diaminotoluene, 2-methyl-1,4-diaminobenzene, 4-methyl-o-phenylenediamine, 2-methoxy-p-phenylenediamine, 2-chloro-1,4-diamino-benzene, 4-amino diphenylamine, 1-amino-4-β-methoxyethylamino-benzene, 1-amino-4-bis-(β-hydroxyethyl)-amonibenzene, 1-3-diamino-benzene, 2-methyl-1,3-diamino-benzene, 2,4-diaminotoluene, 2,6-diaminopyridine, 1-hydroxy-2-amino-benzene, 1-hydroxy-3-amino-benzene, 1-methyl-2-hydroxy-4-amino-benzene, 1-methyl-2-hydroxy-4-β-hydroxyethylamino-benzene, 1-hydroxy-4-amino-benzene, 1-hydroxy-4-methylamino-benzene, 1-methoxy-2,4-diamino-benzene, 1-ethoxy-2,3-diamino-benzene, 1-β-hydroxyethyloxy-2,4-diamino-

benzene, phenazines, such as 4,7-phenazinedicarboxylic acid, 2,7-phenazinedicarboxylic acid, 2-phenazinecarboxylic acid, 2,7-diaminophenazine, 2,8-diaminophenazine, 2,7-diamino-3,8-dimethoxyphenazine, 2,7-diamino-3-methoxyphenazine, 2,7-diamino 3-methoxyphenazine, 3-dimethyl 2,8-phenazinediamine, 2,2'-[(8-amino-7-methyl-2-phenazinyl)imino]bis-ethanol, 2,2'-[(8-amino-7-methoxy-2-phenazinyl)imino]bis-ethanol, 2,2'-[(8-amino-7-chloro-2-phenazinyl)imino]bis-ethanol, 2-[(8-amino-7-methyl-2-phenazinyl)amino]-ethanol, 2,2'-[(8-amino-2-phenazinyl)imino]bis-ethanol, 3-amino-7-(dimethylamino)-2,8-dimethyl-5-phenyl-chloride, 9-(diethylamino)- benzo[a]phenazine-1,5-diol, N-[8-(diethylamino)-2-phenazinyl]- methanesulfonamide, N-(8-methoxy-2-phenazinyl)- Methanesulfonamide, N,N,N',N'-tetramethyl-2,7-phenazinediamine, 3,7-dimethyl-2-phenazinamine, p-amino benzoic acids, such as p-amino benzoic acid ethyl, p-amino benzoic acid glycerid, p-amino benzoic acid isobutyl, p-dimethylamino benzoic acid amil, p-dimethylamino benzoic acid octyl, p-diethoxy amino benzoic amil, p- dipropoxy amino benzoic acid ethyl, acetylsalicylic acid, isatin derivatives, such as 2,3-diamino benzoic acid.

9. The dyeing composition according to claims 8, comprising a dye modifier selected from the group comprising m-phenylenediamine, 2,4-diaminoanisole, 1-hydroxynaphthalene(α-naphthol), 1,4-dihydroxybenzene(hydroquinone), 1,5-dihydroxynapthalene, 1,2-dihydroxybenzene(pyrocatechol), 1,3-dihydroxybenzene (resorcinol), 1,3-dihydroxy-2-methylbenzene, 1,3-dihydroxy-4-chlorobenzene (4-chlororesorcinol), 1,2,3,trihydroxybenzene, 1,2,4-trihydroxybenzene, 1,2,4-trihydroxy-5-methylbenzene, 1,2,4-trihydroxytoluene.

10. A method for dying comprising contacting a laccase derived from a strain of the genus *Scytalidium* with the keratinous fibres and at least one dye precursor in the presence or absence of at least one modifier for a period of time and under conditions sufficient to permit oxidation of the dye precursor into a coloured compound.

11. The method according to claim 10, wherein the dyeing is carried out at a pH in the range from 3.0 to 9.0, preferably 4.0 to 8.0, especially 6.0 to 8.0.

12. Use of an oxidation enzyme derived from a strain of the genus *Scytalidium* for oxidative dyeing keratinous fibres, in particular hair, fur, hide and wool.

13. The use according to claim 14, wherein the oxidation enzyme is derived from a strain of the species *Scytalidium thermophilum*.

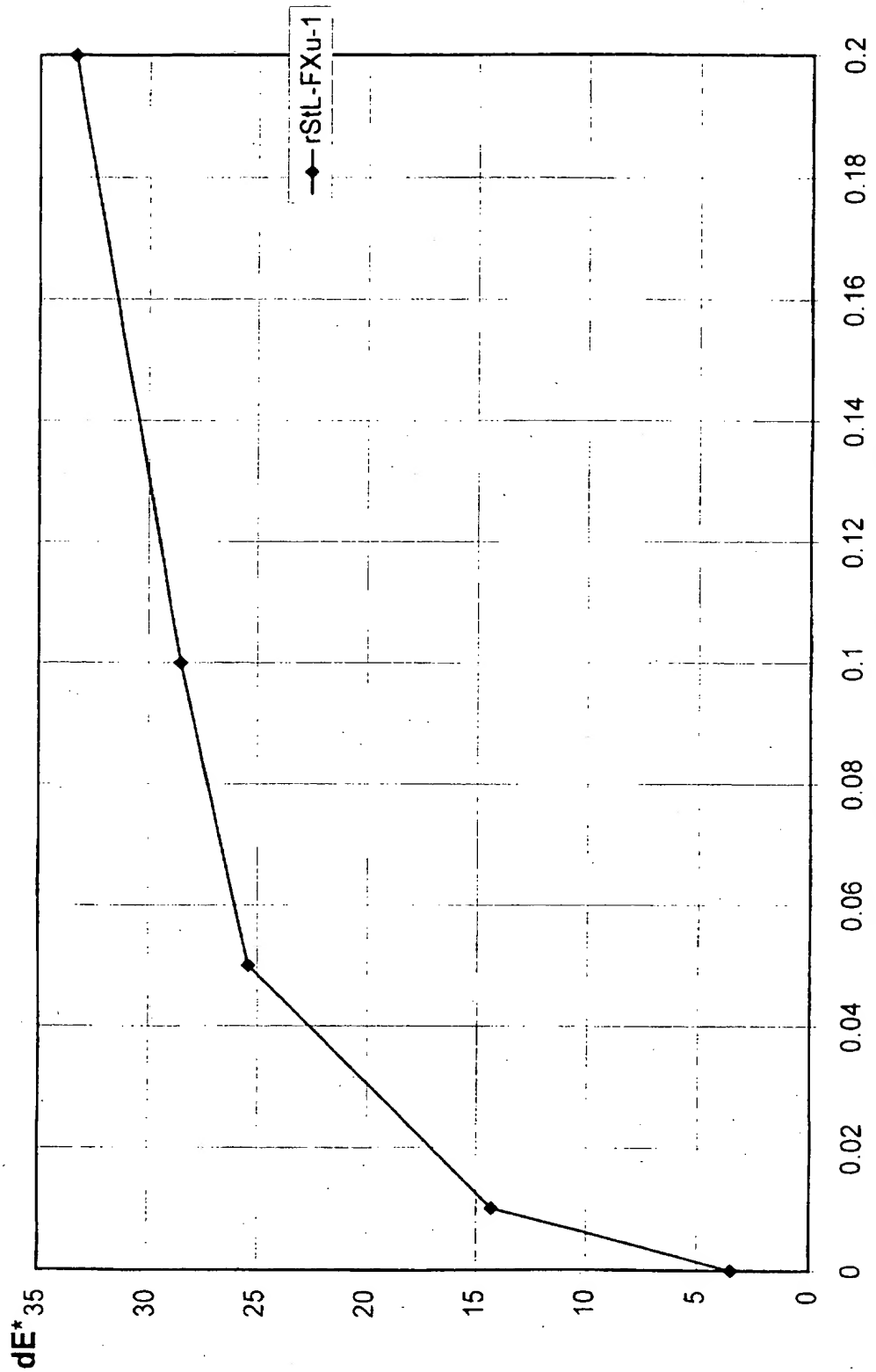

Fig. 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 96/00498

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C09B 67/00, A61K 7/13
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C09B, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 9533837 A1 (NOVO NORDISK BIOTECH, INC.), 14 December 1995 (14.12.95), claims 28, 29; page 15, line 34 - page 16 --	1-13
P,A	WO 9533836 A1 (NOVO NORDISK BIOTECH, INC.), 14 December 1995 (14.12.95), claims 31-42; page 16, line 12 - page 17, line 27; page 34, line 20 - page 36 --	1-13
X	EP 0504005 A1 (PERMA SOCIETE ANONYME), 16 Sept 1992 (16.09.92) --	1-13

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 February 1997	01 -03- 1997
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Gerd Strandell Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 96/00498

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 3251742 A (SAUL SOLOWAY), 17 May 1966 (17.05.66)	1-13
	--	
X	WO 9600290 A1 (NOVO NORDISK BIOTECH, INC.), 4 January 1996 (04.01.96), claims 37-48; page 48, line 25 - page 54, line 24	1-13
	--	
X	STN International, File CAPLUS, CAPLUS accession no. 1991:498981, Saruno, Rinjiro: "Hair- dyeing preparations containing melanin or other polyphenol pigments and manufacture of the pigments"; & JP,A2,910403	1-13
	--	
X	STN International, File CAPLUS, CAPLUS accession no. 1995:974547, Chivukula, Muralikrishna et al: "Phenolic azo dye oxidation by laccase from Pyricularia oryzae"; & Appl. Environ. Microbiol. (1995), 61(12), 4374-77	1-13
	--	
A	DE 4314317 A1 (HENKEL KGAA), 3 November 1994 (03.11.94)	8
	--	
A	WO 9400100 A1 (L'OREAL), 6 January 1994 (06.01.94)	8
	--	
A	WO 9507988 A1 (NOVO NORDISK A/S), 23 March 1995 (23.03.95), claim 41	1-13
	--	

INTERNATIONAL SEARCH REPORT

Information on patent family members

03/02/97

International application No.
PCT/DK 96/00498

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A1- 9533837	14/12/95	AU-A- 2656695	04/01/96
WO-A1- 9533836	14/12/95	AU-A- 2656595	04/01/96
EP-A1- 0504005	16/09/92	AT-T- 121931	15/05/95
		CA-A- 2061826	09/09/92
		DE-D,T- 69202290	09/11/95
		ES-T- 2072720	16/07/95
		FR-A,B- 2673534	11/09/92
		JP-A- 6172145	21/06/94
US-A- 3251742	17/05/66	FR-A- 1363462	00/00/00
		GB-A- 993923	00/00/00
WO-A1- 9600290	04/01/96	AU-A- 2827895	19/01/96
DE-A1- 4314317	03/11/94	EP-A- 0695162	07/02/96
		JP-T- 8509478	08/10/96
		WO-A- 9424988	10/11/94
WO-A1- 9400100	06/01/94	DE-D,T- 69301464	05/06/96
		EP-A,B- 0645999	05/04/95
		FR-A,B- 2692782	31/12/93
		JP-T- 7508271	14/09/95
		US-A- 5538517	23/07/96
WO-A1- 9507988	23/03/95	AU-A- 7833694	03/04/95
		CA-A- 2171288	23/03/95
		CN-A- 1133067	09/10/96
		EP-A- 0719337	03/07/96
		FI-A- 961250	18/03/96
		US-A- 5480801	02/01/96

TRAITE DE COOPERATION EN MATIERE DE BREVETS

PCT

RAPPORT D'EXAMEN PRELIMINAIRE INTERNATIONAL

(article 36 et règle 70 du PCT)

Référence du dossier du déposant ou du mandataire B99/0640QT	**POUR SUITE A DONNER**	voir la notification de transmission du rapport d'examen préliminaire international (formulaire PCT/IPEA/416)
Demande internationale n° PCT/FR00/00456	Date du dépot international *(jour/mois/année)* 24/02/2000	Date de priorité *(jour/mois/année)* 26/03/1999
Classification internationale des brevets (CIB) ou à la fois classification nationale et CIB A61K7/13		
Déposant L'OREAL et al.		

1. Le présent rapport d'examen préliminaire international, établi par l'administaration chargée de l'examen préliminaire international, est transmis au déposant conformément à l'article 36.

2. Ce RAPPORT comprend 6 feuilles, y compris la présente feuille de couverture.

   ☐ Il est accompagné d'ANNEXES, c'est-à-dire de feuilles de la description, des revendications ou des dessins qui ont été modifiées et qui servent de base au présent rapport ou de feuilles contenant des rectifications faites auprès de l'administration chargée de l'examen préliminaire international (voir la règle 70.16 et l'instruction 607 des Instructions administratives du PCT).

   Ces annexes comprennent feuilles.

3. Le présent rapport contient des indications relatives aux points suivants:

I	☒	Base du rapport
II	☐	Priorité
III	☐	Absence de formulation d'opinion quant à la nouveauté, l'activité inventive et la possibilité d'application industrielle
IV	☐	Absence d'unité de l'invention
V	☒	Déclaration motivée selon l'article 35(2) quant à la nouveauté, l'activité inventive et la possibilité d'application industrielle; citations et explications à l'appui de cette déclaration
VI	☐	Certains documents cités
VII	☒	Irrégularités dans la demande internationale
VIII	☒	Observations relatives à la demande internationale

Date de présentation de la demande d'examen préliminaire internationale	Date d'achèvement du présent rapport
24/10/2000	29.03.2001
Nom et adresse postale de l'administration chargée de l'examen préliminaire international: Office européen des brevets D-80298 Munich Tél. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Fonctionnaire autorisé Culmann, J-C N° de téléphone +49 89 2399 8487

Formulaire PCT/IPEA/409 (feuille de couverture) (janvier 1994)

RAPPORT D'EXAMEN PRÉLIMINAIRE INTERNATIONAL

Demande internationale n° PCT/FR00/00456

I. Base du rapport

1. Ce rapport a été rédigé sur la base des éléments ci-après (*les feuilles de remplacement qui ont été remises à l'office récepteur en réponse à une invitation faite conformément à l'article 14 sont considérées dans le présent rapport comme "initialement déposées" et ne sont pas jointes en annexe au rapport puisqu'elles ne contiennent pas de modifications (règles 70.16 et 70.17).*) :

Description, pages:

1-17 version initiale

Revendications, N°:

1-26 version initiale

2. En ce qui concerne la **langue**, tous les éléments indiqués ci-dessus étaient à la disposition de l'administration ou lui ont été remis dans la langue dans laquelle la demande internationale a été déposée, sauf indication contraire donnée sous ce point.

   Ces éléments étaient à la disposition de l'administration ou lui ont été remis dans la langue suivante: , qui est :

   ☐ la langue d'une traduction remise aux fins de la recherche internationale (selon la règle 23.1(b)).
   ☐ la langue de publication de la demande internationale (selon la règle 48.3(b)).
   ☐ la langue de la traduction remise aux fins de l'examen préliminaire internationale (selon la règle 55.2 ou 55.3).

3. En ce qui concerne les **séquences de nucléotides ou d'acide aminés** divulguées dans la demande internationale (le cas échéant), l'examen préliminaire internationale a été effectué sur la base du listage des séquences :

   ☐ contenu dans la demande internationale, sous forme écrite.
   ☐ déposé avec la demande internationale, sous forme déchiffrable par ordinateur.
   ☐ remis ultérieurement à l'administration, sous forme écrite.
   ☐ remis ultérieurement à l'administration, sous forme déchiffrable par ordinateur.
   ☐ La déclaration, selon laquelle le listage des séquences par écrit et fourni ultérieurement ne va pas au-delà de la divulgation faite dans la demande telle que déposée, a été fournie.
   ☐ La déclaration, selon laquelle les informations enregistrées sous déchiffrable par ordinateur sont identiques à celles du listages des séquences Présenté par écrit, a été fournie.

4. Les modifications ont entraîné l'annulation :

   ☐ de la description, pages :
   ☐ des revendications, n^{os} :
   ☐ des dessins, feuilles :

THIS PAGE BLANK

5. ☐ Le présent rapport a été formulé abstraction faite (de certaines) des modifications, qui ont été considérées comme allant au-delà de l'exposé de l'invention tel qu'il a été déposé, comme il est indiqué ci-après (règle 70.2(c)) :

   (Toute feuille de remplacement comportant des modifications de cette nature doit être indiquée au point 1 et annexée au présent rapport)

6. Observations complémentaires, le cas échéant :

V. Déclaration motivée selon l'article 35(2) quant à la nouveauté, l'activité inventive et la possibilité d'application industrielle; citations et explications à l'appui de cette déclaration

1. Déclaration

Nouveauté	Oui :	Revendications	1-26
	Non :	Revendications	
Activité inventive	Oui :	Revendications	1-26
	Non :	Revendications	
Possibilité d'application industrielle	Oui :	Revendications	1-26
	Non :	Revendications	

2. Citations et explications
   voir feuille séparée

VII. Irrégularités dans la demande internationale

Les irrégularités suivantes, concernant la forme ou le contenu de la demande internationale, ont été constatées :
voir feuille séparée

VIII. Observations relatives à la demande internationale

Les observations suivantes sont faites au sujet de la clarté des revendications, de la description et des dessins et de la question de savoir si les revendications se fondent entièrement sur la description :
voir feuille séparée

THIS PAGE BLANK

V Déclaration motivée selon l'article 35(2) quant à la nouveauté, l'activité inventive et la possibilité d'application industrielle; citations et explications à l'appui de cette déclaration

1. Le document EP-A-0 716 846 semble être le plus pertinent parmi les documents cités dans le rapport de recherche.

   Il vise une composition pour la teinture des cheveux, basée sur l'emploi d'une enzyme oxydante, l'uricase (à l'exception de tout autre enzyme), en combinaison avec une base d'oxydation et l'acide urique. Un agent réducteur, dont le potentiel redox doit être plus positif que celui de l'acide ascorbique tout en étant plus négatif que celui de l'acide urique, peut être présent, comme la N-acétyl cystéine.

   Cet agent réducteur a une action anti-oxydante sans toutefois inhiber les réactions nécessaires à l'obtention d'une teinture.

2. La présente demande diffère de cet art antérieur en ce que:

   - l'enzyme oxydante est une laccase;
   - sans être en présence d'un substrat apparenté.

3. Dans la mesure où cet art antérieur:

   - n'enseigne ni ne suggère l'emploi d'une laccase en lieu et place d'une uricase;
   - et où le choix de l'agent réducteur est déterminé par le potentiel d'oxydo-réduction du substrat apparenté à l'enzyme

   la présente demande, basée sur l'emploi et d'une laccase et de la N-acétyl cystéine ne peut être considéré comme découlant de façon évidente du document FR-A-0 716 846.

   A moins de considérer que l'uricase peut être substituée par une laccase, mais

que la N-acétyl cystéine doit toutefois être conservée; ce qui est i) en flagrante contradiction avec l'enseignement de ce document (seule l'uricase est citée) et ii) illogique (le choix de la N-acétyl cystéine est fonction du potentiel du substrat associé à l'enzyme choisie). Ce que ne peut faire la présente administration.

4. Le but poursuivi par la demanderesse, à savoir la préservation des précurseurs de colorants d'oxydation et des coupleurs, sans inhiber l'action des laccases, est atteint, et de façon convaincante (voir les résultats expérimentaux).

5. La présente administration considère donc que l'objectif fixé par la demanderesse est atteint d'une façon originale et qui n'est pas déductible de l'art antérieur; les conditions énoncées à l'article 33(3) du PCT semblent donc être satisfaites.

VII Irrégularités dans la demande internationale

Contrairement à l'énoncé de la règle 27 (1) b) CBE, la demanderesse n'a ni cité ni commenté dans la description le document EP-A-0 716 846.

VIII Observations relatives à la demande internationale

La revendication 2 précise qu'au moins une laccase est incorporée dans la composition (A), alors que la description (à la page 3, ligne 17) ne fait référence qu'à l'incorporation d'une laccase.

Dans les revendications 17 et 18, les polymères ont des définitions plus larges que celle trouvées dans la description (cf. le poids moléculaire à la page 11, les lignes 3 et 19); les définitions des revendications ne sont donc pas en accord avec la description.

Le libellé des revendications 21 et 22 manque de clarté dans la mesure où ces revendications se réfèrent à une partie du contenu de revendications de procédé; il eut été préférable d'adopter un libellé extensif de l'objet qui y est revendiqué, même si une telle formulation serait apparemment plus "lourde".

La revendication 23 est indépendante, mais concerne en fait une étape du procédé revendiqué à la revendication 2; dans la mesure où un nombre excessif de revendication du même type est à éviter, cette revendication aurait du être reformulée comme une revendication dépendante.

Le libellé de la revendication 24 est susceptible d'être plus clair, car il est de fait très proche du libellé de la revendication 2, et n'est caractérisé que par le fait que la composition (A) est stocké séparément (ce qui est déjà évoqué à la revendication 2 !